

Claims

What is claimed is:

- 5 1. A method of screening compounds suspected of being skin irritants, comprising:
- a) providing
- i) a compound suspected of being a skin irritant;
- ii) a skin equivalent comprising a irritant responsive reporter
- 10 gene construct; and
- b) contacting said skin equivalent with said compound; and
- c) measuring the level of gene expression from said reporter gene construct.
- 15 2. The method of Claim 1, wherein said human skin equivalent has a surface electrical capacitance of from about 80 to about 120 pF.
3. The method of Claim 1, wherein the content of ceramides 5, 6, and 7 in said skin equivalent is from about 20 to about 50% of total ceramide content.
- 20 4. The method of Claim 1, wherein the content of ceramide 2 in said skin equivalent is from about 10 to about 40% of total ceramide content.
5. The method of Claim 1, wherein said skin equivalent comprises
- 25 keratinocytes selected from the group consisting of primary keratinocytes and immortalized keratinocytes.
6. The method of Claim 5, wherein said immortalized keratinocytes are NIKS cells.

7. The method of Claim 1, wherein said keratinocytes express heterologous GKLF.

8. The method of Claim 1, wherein said irritant responsive reporter construct comprises at least a portion of a gene selected from the group consisting of interleukin-8 and interleukin-1 α .

9. The method of Claim 8, wherein said portion comprises a regulatory region.

10. A composition comprising NIKS cells, said NIKS cells comprising an irritant responsive reporter gene construct.

11. The composition of Claim 10, said NIKS cells having a surface electrical capacitance of from about 40 to about 240 pF.

12. The composition of Claim 10, said NIKS cells having a surface electrical capacitance of from about 80 to about 120 pF.

13. The composition of Claim 10, wherein the combined content of ceramides 5, 6, and 7 in said NIKS cells is from about 20 to about 50% of total ceramide content.

14. The composition of Claim 10, wherein the content of ceramide 2 in said NIKS cells is from about 10 to about 40% of total ceramide content.

15. The composition of Claim 10, wherein said NIKS cells express heterologous GKLF.

16. The NIKS cells of Claim 10, further comprising keratinocytes derived from two different sources.

17. The NIKS cells of Claim 10, further comprising a DNA construct comprising a sequence encoding GKLf operably linked to an exogenous promoter.

5 18. An organotypic culture comprising the NIKS cells of Claim 10.

19. A method of identifying irritant responsive genes, comprising:

a) providing

i) a skin irritant compound;

10 ii) a gene expression array; and

iii) a skin equivalent; and

b) contacting said skin irritant compound with said skin equivalent to generate irritant treated skin equivalent;

15 c) isolating a first mRNA sample from said irritant treated skin equivalent and a second mRNA sample from said skin equivalent;

d) contacting said first and second mRNA samples with said gene expression array;

20 e) analyzing said gene expression array under conditions such that the identity of genes that are expressed at greater levels in said irritant treated skin equivalent than in said skin equivalent are determined.

20. The method of Claim 19, wherein skin equivalent comprises cultured human keratinocytes.

25 21. The method of Claim 20, wherein said cultured human keratinocytes are present as an organotypic culture.

30 22. The method of Claim 19, wherein said irritant responsive reporter construct comprises at least a portion of a gene selected from the group consisting of interleukin-8 and interleukin-1 α .

23. The method of Claim 22, wherein said portion comprises a regulatory region.

5 24. The method of Claim 19, wherein said gene array comprises human cDNA sequences.

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